

We claim:

1. A method of preparing a stabilized glycopeptide antibiotic conjugate, comprising:

(a) forming a glycopeptide antibiotic conjugate comprising a glycopeptide antibiotic member bound to a ligand, said ligand being bound to a ligand-binding partner immobilized on a solid support, wherein said conjugate is formed under conditions that minimize intermolecular hydrogen bonding between said glycopeptide antibiotic members;

(b) mixing said conjugate with at least one stabilizing agent that prevents dimerization of said glycopeptide antibiotic member, wherein the pH of the mixture is between about pH 7 to pH 9;

(c) heating the mixture at a temperature between about 40° and 50° C for about 3 to 14 days to ensure colloidal stability; and

(d) storing the heat-treated mixture obtained in step (c) in a diluent buffer having a pH of about 6.5 to 8.5

2. The method of claim 1, wherein said antibiotic is selected from the group consisting of vancomycin, eremomycin, and ristocetin A.

3. The method of claim 1, wherein said antibiotic is vancomycin.

4. The method of claim 1, wherein the stabilizing agent is selected from the group consisting of N α ,N β -diacetyl-L-lysine-D-alanine-D-alanine, heparin, acteyl-D-alanine-D-alanine and acetyl-D-alanine-D-alanine-D-alanine.

5. The method of claim 1, wherein step (a) further comprises:

(i) coupling said ligand to said immobilized ligand-binding partner to form a glycopeptide antibiotic conjugate under alkaline conditions that prevent aggregation of the glycopeptide antibiotic conjugate; and

(ii) isolating said glycopeptide antibiotic conjugate under alkaline conditions that minimize dimerization of said glycopeptide antibiotic member.

6. The method of claim 5, wherein step (i) is performed at about pH 9.

7. The method of claim 5, wherein step (ii) is performed at about pH 9.

8. The method of claim 5, wherein said glycopeptide antibiotic conjugate is isolated by diafiltration, dialysis or size exclusion chromatography.

9. The method of claim 1, wherein said solid support is carrier particles selected from the group consisting of latex particles, metallic particles, colloidal metals and colloidal metal oxides..

10. The method of claim 9, wherein said carrier particles are latex particles.

11. The method of claim 10, wherein said latex particles having said ligand binding partner bound thereto are heat-stressed prior to coupling to said bidentate.

12. The method of claim 1, wherein said ligand is biotin.

13. The method of claim 12, wherein said ligand-binding partner is selected from the group consisting of avidin, streptavidin, and an anti-biotin antibody.

14. A stabilized glycopeptide antibiotic conjugate prepared according to the method of claim 1.

15. The stabilized glycopeptide antibiotic conjugate of claim 14, wherein said conjugate has a heat-stressed stability of about seven days at about 45° C.

16. The conjugate of claim 14, wherein said glycopeptide antibiotic conjugate has a shelf life of about eighteen months at about 4° C.

17. The conjugate of claim 14, wherein said glycopeptide antibiotic conjugate has an on-instrument stability of 53 days.

18. A stabilized glycopeptide antibiotic conjugate formulation, comprising:

a) a bidentate conjugate comprising a glycopeptide antibiotic member bound to a ligand, said ligand being bound to a ligand-binding partner immobilized on a solid support; and

b) at least one stabilizing agent that prevents dimerization of said glycopeptide antibiotic member, wherein said bidentate conjugate and said stabilizing agent are dissolved in a diluent buffer having a neutral pH.

19. The method of claim 18, wherein said glycopeptide antibiotic is selected from the group consisting of vancomycin, eremomycin, and ristocetin A.

20. The method of claim 17, wherein said antibiotic is vancomycin.

21. The stabilized glycopeptide antibiotic conjugate formulation of claim 18, wherein
5 said stabilizing agent is selected from the group consisting of $N\alpha,N\beta$ -diacetyl-L-lysine-D-alanine-D-alanine, heparin, acteyl-D-alanine-D-alanine and acetyl-D-alanine-D-alanine-D-alanine.

22. The stabilized glycopeptide antibiotic conjugate formulation of claim 18, wherein
10 said solid support is a carrier particle selected from the group consisting of latex particles, metallic particles, colloidal metals and colloidal metal oxides.

23. The stabilized glycopeptide antibiotic conjugate formulation of claim 22, wherein
said carrier particles are latex particles.

24. The stabilized glycopeptide antibiotic conjugate formulation of claim 23, wherein
15 said latex particles having said ligand binding partner immobilized thereon are heat-stressed.

25. The stabilized glycopeptide antibiotic conjugate formulation of claim 18, wherein
said ligand is biotin.

26. The stabilized glycopeptide antibiotic conjugate formulation of claim 25, wherein
20 said ligand-binding partner is selected from the group consisting of avidin, streptavidin, and an anti-biotin antibody.

27. The stabilized glycopeptide antibiotic conjugate formulation of claim 18, further
comprising one or more rate enhancers selected from the group consisting of
ethylenediamine, polyethylene glycol, 1,3 diaminopropane and 1,2-diaminopropane.

28. A stabilized vancomycin conjugate formulation for use in a homogeneous assay of
25 vancomycin in a test sample, comprising a biotinylated vancomycin bound to a biotin-binding partner, said biotin-binding partner being immobilized on a solid support,

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wherein said vancomycin conjugate has a heat-stressed stability of at least 7 days at 45° C or a shelf life of at least 18 months at 4° C.

29. The formulation of claim 28, further comprising at least one stabilizing agent that prevents dimerization of said vancomycin member.

5 30. The formulation of claim 29, wherein said stabilizing agent is selected from the group consisting of N α ,N β -diacetyl-L-lysine-D-alanine-D-alanine, heparin, acteyl-D-alanine-D-alanine and acetyl-D-alanine-D-alanine-D-alanine.

31. An assay for determining the presence of a glycopeptide antibiotic in a test sample, said method comprising:

- 10 (a) providing a stabilized glycopeptide antibiotic conjugate formulation comprising:
- (i) a bidentate conjugate comprising a glycopeptide antibiotic member bound to a ligand, said ligand being bound to a ligand-binding partner immobilized on a solid support; and
 - 15 (ii) at least one stabilizing agent that prevents dimerization of said glycopeptide antibiotic member, wherein said bidentate conjugate and said stabilizing agent are dissolved in a conjugate diluent having a neutral pH;
 - (b) providing an antibody that is immunoreactive with said glycopeptide antibiotic;
 - 20 (c) mixing said sample with said glycopeptide antibiotic conjugate formulation and said antibody to form a reaction mixture;
 - (d) incubating said reaction mixture under conditions that allow binding of said antibody to said glycopeptide antibiotic contained in the sample or said glycopeptide antibiotic member of said bidentate conjugate; and
 - 25 (e) determining the amount of said glycopeptide antibiotic member of said bidentate conjugate that bound with said antibody, wherein the amount is inversely proportional to the concentration of said glycopeptide antibiotic in said sample.

32. The assay of claim 31, wherein said glycopeptide antibiotic is selected from the group consisting of vancomycin, eremomycin, and ristocetin A.

30 33. The assay of claim 31, wherein said antibiotic is vancomycin.

34. The assay of claim 31, wherein step (c) further comprises contacting the sample with said antibody to form a mixture, and then contacting the mixture with said glycopeptide antibiotic conjugate formulation.

35. The assay of claim 31, wherein step (c) further comprises contacting the sample with said glycopeptide antibiotic conjugate formulation to form a mixture, and then contacting the mixture with said antibody.

36. The assay of claim 31, wherein said stabilizing agent is selected from the group consisting of $N\alpha,N\beta$ -diacetyl-L-lysine-D-alanine-D-alanine, heparin, acetyl-D-alanine-D-alanine and acetyl-D-alanine-D-alanine-D-alanine.

37. The assay of claim 31, further comprising adding one or more rate enhancers to said reaction mixture to enhance the binding of said antibody to said glycopeptide antibiotic in said sample or to said glycopeptide antibiotic member.

38. The assay of claim 37, wherein said rate enhancer is selected from the group consisting of ethylenediamine, polyethylene glycol, 1,3 diaminopropane and 1,2-diaminopropane.

39. The assay of claim 37, wherein said one or more rate enhancers are added to the glycopeptide antibiotic conjugate formulation.

40. The assay of claim 37, wherein said one or more rate enhancers is added in step (c).

41. The assay of claim 31, wherein said conjugate diluent is a Tris buffer, a phosphate buffer or a borate buffer.

42. The assay of claim 31, further comprising adding a dose response modulator to said reaction mixture.

43. The assay of claim 42, wherein said dose response modulator is selected from the group consisting of ethylenediamine, 1,2-diaminopropane, 1,3-diaminopropane, $N\alpha,N\epsilon$ -diacetyl-D-alanine-D-alanine-L-lysine and detergents.

44. The assay of claim 31, wherein said solid support is a carrier particle selected from the group consisting of latex particles, metallic particles colloidal metals, colloidal and metal oxides.

45. The assay of claim 44, wherein said carrier particle is a latex particle.

5 46. The assay of claim 45, wherein said latex particles having said ligand binding partner immobilized thereon are heat-stressed prior.

47. The assay of claim 31, wherein said ligand is biotin.

48. The assay of claim 47, wherein said ligand-binding partner is selected from the group consisting of avidin, streptavidin, and an anti-biotin antibody.

10 49. The assay of claim 31, wherein the amount of bidentate conjugate that bound with said antibody is determined using nephelometric or turbidimetric means.

50. A test kit for detecting the presence of a glycopeptide antibiotic in a test sample, comprising:

- a) an assay medium;
- 15 b) a stabilized glycopeptide antibiotic conjugate formulation, comprising:
 - (i) a bidentate conjugate comprising a glycopeptide antibiotic member bound to a ligand, said ligand being bound to a ligand-binding partner immobilized on a solid support; and
 - (ii) at least one stabilizing agent that prevents dimerization of said
- 20 glycopeptide antibiotic member, wherein said bidentate conjugate and said stabilizing agent are dissolved in a conjugate diluent having a neutral pH; and
- c) an anti- glycopeptide antibiotic antibody.

51. The test kit of claim 50, wherein said glycopeptide antibiotic is selected from the group consisting of vancomycin, eremomycin, and ristocetin A.

25 52. The test kit of claim 50, wherein said glycopeptide antibiotic is vancomycin.

53. The test kit of claim 50, wherein said stabilizing agent is selected from the group consisting of N α ,N ϵ -diacetyl-D-alanine-D-alanine-L-lysine, heparin, acteyl-D-alanine-D-alanine and acetyl-D-alanine-D-alanine-D-alanine.

54. The test kit of claim 50, further comprising one or more rate enhancers to enhance
5 the binding of said antibody to said glycopeptide antibiotic in said sample or to said glycopeptide antibiotic member.

55. The test kit of claim 54, wherein said rate enhancer is selected from the group consisting of a lower molecular weight amine and a mixture of a lower molecular weight amine and polyethylene glycol.

10 56. The test kit of claim 54, wherein said lower molecular weight amine is selected from the group consisting of ethylenediamine, 1,2-diaminopropane and 1,3-diaminopropane.

57. The test kit of claim 50, further comprising a dose response modulator.

15 58. The test kit of claim 57, wherein said dose response modulator is selected from the group consisting of ethylenediamine, 1,2-diaminopropane, 1,3-diaminopropane, N α ,N ϵ -diacetyl-D-alanine-D-alanine-L-lysine and a detergent.

59. The test kit of claim 50, wherein said ligand is biotin.

60. The test kit of claim 59, wherein said ligand-binding partner is selected from the group consisting of avidin, streptavidin, and an anti-biotin antibody.

20 61. The test kit of claim 50, wherein said assay medium is a TRIS buffer, a phosphate buffer, or a borate buffer.

62. The test kit of claim 50, wherein said solid support is carrier particles selected from the group consisting of latex particles, metallic particles, colloidal metals and colloidal metal oxides.

25 63. The test kit of claim 62, wherein said carrier particles are latex particles.

64. The test kit of claim 62, wherein said latex particles having said ligand binding partner immobilized thereon are heat-stressed.

65. A method of enhancing the rate of immune complex formation in a particle-based immunoassay for the detection of an analyte in a test sample, wherein the immunoassay

5 comprises:

- (a) providing an anti-analyte antibody;
- (b) providing a solid support bound analyte;
- (c) providing one or more rate enhancers; and
- (d) combining said test sample, said antibody, said support bound analyte, and

10 one or more rate enhancers under conditions that allow binding of said antibody to said analyte in said test sample or said support-bound analyte, wherein said one or more rate enhancers enhance the binding of said antibody to said analyte.

66. The method of claim 65, wherein said rate enhancer is selected from the group consisting of a lower molecular weight amine and a mixture of a lower molecular weight
15 amine and polyethylene glycol.

67. The method of claim 66, wherein said lower molecular weight amine is selected from the group consisting of ethylenediamine, 1,2-diaminopropane and 1,3-diaminopropane.

68. The method of claim 65, wherein said solid support analyte comprises an analyte
20 bound to a ligand, wherein said ligand is coupled to a ligand-binding member immobilized said a solid support.

69. The method of claim 68, wherein said analyte is vancomycin.

70. The method of claim 69, wherein said ligand is biotin.

71. The method of claim 70, wherein said ligand-binding partner is selected from the
25 group consisting of avidin, streptavidin, and an anti-biotin antibody.

72. The method of claim 65, wherein said solid support is a carrier particle selected from the group consisting of latex particles, metallic particles colloidal metals, colloidal and metal oxides.

73. A method of modulating the dose response in a particle-based immunoassay for the detection of an analyte in a test sample, wherein the immunoassay comprises:

- (a) providing an anti-analyte antibody;
- (b) providing a solid support bound analyte;
- (c) providing one or more dose response modulators; and
- (d) combining said test sample, said antibody, said support bound analyte, and

one or more dose response modulators under conditions that allow binding of said antibody to said analyte in said test sample or said support-bound analyte.

74. The method of claim 73, wherein said dose response modulator is selected from the group consisting of ethylenediamine, 1,2-diaminopropane 1,3-diaminopropane and N α ,N ϵ -diacetyl-D-alanine-D-alanine-L-lysine.